Exact Scientific Services
www.exactscientific.com

1355 Pacific Place
Suite 101
Ferndale, WA 98248
phone: 360.733.1205
fax: 888-818-2978
email: lab@exactscientific.com

AOAC Use Dilution Study Final Report

By
Katherine Sandoval
&
Kent Oostra

For

Synergy Americas, Inc
127 Stream Road
Winterport, ME 04496
Reference Nu: 21-13177

Copyright © 2021
Revision 1.0 10/12/2021
Exact Scientific Services, Inc.
1355 Pacific Place
Suite 101
Ferndale, WA 98248
360.733.1205

The following is confidential information. This report is not to be reproduced or disseminated in any fashion without prior written approval from
Exact Scientific Services, Inc and/or Synergy Americas, Inc.
All rights reserved.

Exact Scientific Services
www.exactscientific.com
1355 Pacific Place
Suite 101
Ferndale, WA 98248
phone: 360.733.1205
fax: 888-818-2978
email: lab@exactscientific.com

FINAL STUDY REPORT SUMMARY

Study Title
AOAC Use-Dilution Test

Product Identity
Synergy Envirotab 4g / 400ppm in 1liter of water
Lots: 2020/12-05, 2020/12-06, 2020/12-07
Final Concentration at 3% LCL: 388ppm

Test Microorganisms
Staphylococcus aureus ATCC 6538
Trichophton interdigitale ATCC 9533

Test Guidelines
EPA Guideline 8.10.2000
EPA 2018 Guideline 810.2200 (D)(3)

Data Requirements
U.S. EPA OCSPP 810.2200

Author
Katherine Sandoval
Molecular Biology Director

Study Completion Date
08/09/2021

Performing Laboratory
Exact Scientific Services, Inc.
1355 Pacific Place, Suite 101
Ferndale, WA 98248

Study Sponsor
Michael R. Martin
Synergy Americas, Inc.
127 Stream Road
Winterport, Maine 04496

Laboratory Reference Number
21-13177 53261

Exact Scientific Services
www.exactscientific.com

1355 Pacific Place
Suite 101
Ferndale, WA 98248
phone: 360.733.1205
fax: 888-818-2978
email: lab@exactscientific.com

STATEMENT OF NO DATA CONFIDENTIALITY

No claim of confidentiality, on any basis whatsoever, is made for any information contained in this document. I acknowledge that information not designated as within the scope of FIFRA section 10(d)(1)(A), (B), or (C) and which pertains to a registered or previously registered pesticide is not entitled to confidential treatment and may be released to the public, subject to the provisions regarding disclosure to multinational entities under FIFRA 10(g).

Company: Exact Scientific Services, Inc.

Agent/Submitter: Katherine Sandoval

Title: Director of Molecular Biology

Date: October 12, 2021

Signature: Katherine Sandoval

Exact Scientific Services
www.exactscientific.com
1355 Pacific Place
Suite 101
Ferndale, WA 98248
phone: 360.733.1205
fax: 888-818-2978
email: lab@exactscientific.com

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

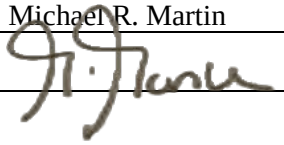
The following is a detailed description of all differences between the practices used in the study and those required by 40 CFR part § 160.

Records concerning test substance characteristics (i.e. composition, purity, stability, strength, solubility) are maintained by the Study Sponsor.

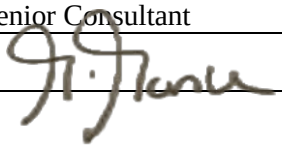
Study Director

Company:	Exact Scientific Services, Inc.		
Name:	Kent Oostra		
Title:	CEO		
Signature:	Kent Oostra	Date:	1/20/23

Study Sponsor

Company:	Synergy Americas, Inc.		
Name:	Michael R. Martin		
Signature:		Date:	1/25/2023

Study Submitter

Company:	Synergy Americas, Inc.		
Name:	Michael R. Martin		
Title:	Senior Consultant		
Signature:		Date:	1/25/2023

1355 Pacific Place
Suite 101
Ferndale, WA 98248
phone: 360.733.1205
fax: 888-818-2978
email: lab@exactscientific.com

QUALITY ASSURANCE STATEMENT

The following quality assurance audits were conducted in accordance with Good Laboratory Practice Standards outlined in 40 CFR § 160 and reported to management and the Study Director:

Phase Inspected	Date Inspected	Date Reported to Study Director	Date Reported to Management
Protocol	06/21/2021	06/22/2021	06/22/2021
In Process (Test)	06/24/2021	08/13/2021	08/30/2021
Draft Report	08/30/2021	08/31/2021	08/31/2021
Final Report	08/31/2021	10/12/2021	11/01/2021

1355 Pacific Place
Suite 101
Ferndale, WA 98248
phone: 360.733.1205
fax: 888-818-2978
email: lab@exactscientific.com

PERSONNEL INVOLVED IN THE STUDY

Analyst

Name: Joleen Gouette

Title: Molecular Biology Technician

Study Director

Name: Katherine Sandoval

Title: Director of Molecular Biology

Professional or Supervisory Personnel

Name: Kent Oostra

Title: CEO

1355 Pacific Place
Suite 101
Ferndale, WA 98248
phone: 360.733.1205
fax: 888-818-2978
email: lab@exactscientific.com

TABLE OF CONTENTS

Exact Scientific Services
www.exactscientific.com
1355 Pacific Place
Suite 101
Ferndale, WA 98248
phone: 360.733.1205
fax: 888-818-2978
email: lab@exactscientific.com

FINAL STUDY REPORT SUMMARY

Study Title:	AOAC Use-Dilution Test
Study Identification Number:	21-13177 53261
Test Method:	ESS 6.2.54; AOAC Official Method 955.15
Test Microorganisms:	*Staphylococcus aureus* ATCC 6538 *Trichophyton interdigitale* ATCC 9533
Test Substance:	Synergy Envirotab Lots: 2020/12-05, 2020/12/06, 2020/12-07
Test Substance Dilution:	1 tablet dissolved in 1030mL AOAC Synthetic Hard Water Final concentration 388 ppm
Carrier Type:	Polished stainless-steel cylinders; type 304 stainless steel; Fisher Scientific catalog number 079075Q
Number of Carriers Per Lot:	*Staphylococcus aureus*: 60 *Trichophnyton interdigitale*: 10
Contact Time:	4 Minutes
Exposure Temperature:	20±1ºC
Neutralizer:	Letheen Broth containing 19.4% Polysorbate 80
Study Objective:	This test is designed to determine effectiveness for a product to be used as a disinfectant. It measures the potential of the test material to disinfect hard surfaces contaminated with bacteria and/or fungi.
Study Dates:	Study Initiation:06/07/2021 Experimental Start: 06/24/2021 Experimental End: 08/04/2021 Study Completion: 08/13/2021
Media and Reagents:	Deionized Water AOAC Synthetic Hard Water Sodium Hydroxide Solution, 1N (NaOH) Phosphate Buffered Dilution Water (PBW) Letheen Broth (LB) 3M Rapid Aerobic Count Petrifilm 3M Yeast/Mold Petrifilm Sabbourd Dextrose Agar (SabDex) Blood Agar with Esculin (BEA) Tryptic Soy Broth (TSB) Baird Parker Agar (BPA)

1355 Pacific Place
Suite 101
Ferndale, WA 98248
phone: 360.733.1205
fax: 888-818-2978
email: lab@exactscientific.com

Gram Stain Reagents
Methylene Blue Stain

Test Synopsis: Specified number of carriers per organism per test lot were inoculated with a known concentration of microorganism. Carriers were treated with test substance for client specified contact time. Carriers were neutralized after exposure to test substance. Neutralizing broth with carriers was incubated to determine effectiveness of test substance.

Study Results:

Test Date	Microorganism	Number Confirmed Positive Carriers/Total Number Tested			Carrier Control Mean Log Density
		Lot: 2020/12-05	Lot: 2020/12-06	Lot: 2020/12-07	
7/7/2021	*T. interdigitale*	N/A	0/10	N/A	5.20×10^6
7/8/2021	*T. interdigitale*	0/10	N/A	N/A	5.50×10^6
7/26/2021	*S. aureus*	1/60	N/A	N/A	1.00×10^7
7/28/2021	*S. aureus*	N/A	0/60	N/A	7.10×10^6
8/2/2021	*S. aureus*	N/A	N/A	2/60	8.80×10^6

1355 Pacific Place
Suite 101
Ferndale, WA 98248
phone: 360.733.1205
fax: 888-818-2978
email: lab@exactscientific.com

TEST PROCEDURES

Test Substance Preparation

Test substance was prepared per submitter instructions:

Prepare a 1000mgL^{-1} Chlorine Dioxide Charge Solution, reduced to LCL of 3% reduction diluted in sterile AOAC Hard Water (AHW). See calculations below:

i 400 ppm solution x 0.97 LCL concentration = 388 ppm

Equation: C1V1=C2V2

400 ppm * 1000 mL AHW = 388 ppm * X mL AHW

$$\frac{400 * 1000}{388} = 1031 \text{ mL}$$

ii Dissolve one pouch in 1031 mL AHW water with little to no headspace.
iii Allow tablet to fully dissolve approximately 1 hour.
iv Invert container several times to ensure complete mixing.
v Store in a cool, dark environment. Use within 3 hours of preparation.
vi Test with exposure time of 4 minutes.

Use Dilution Method Procedures

See appendix 1: ESS 6.1.52

PROTOCOL AMENDMENTS

None. Initial version performed.

PROTOCOL DEVIATIONS

EPA Use Dilution Protocol was followed directly with the following exceptions.

i All results were quantified in tandem with the qualitative results provided by the method. All sample tubes were sonicated and plated in the same manner as the pre and post control carriers to estimate the kill rate of the product.
ii Sterile, disposable pipettes were used to transfer carriers rather than sterile wire loops to prevent cross contamination.
iii Circulating chiller was not used during protocol. Temperature was maintained at 24±2°C in ambient conditions.

STUDY CONTROLS

Carrier Sterility Control:

For each test lot, an uninoculated carrier was collected and incubated in 10mL of letheen broth alongside all test carriers.

Viability Control:

For each test lot, an inoculated, untreated carrier was collected and incubated in 10mL of letheen broth alongside all test carriers.

1355 Pacific Place
Suite 101
Ferndale, WA 98248
phone: 360.733.1205
fax: 888-818-2978
email: lab@exactscientific.com

Negative Process Control:
For each test lot, an uninoculated carrier was treated alongside test carriers to ensure no contamination occurred during any test procedures.

Neutralization Subculture Sterility Controls:
Four primary neutralizing subculture media tubes and four secondary neutralizing subculture media tubes were incubated alongside test carriers.

Media Sterility Controls:
All media lots went through quality control testing including inoculation with test organisms to ensure growth promotion, sterility evaluation, and pH monitoring.

Test Microorganism Purity Control:
Test cultures used in this study were sub-cultured onto blood agar (*S. aureus)* or SabDex agar (*T. interdigitale)* and visually examined for purity prior to evaluation.

Confirmation of Positive:
Positive tubes with turbidity/growth were confirmed via microscopic exam and/or colony morphology on selective agar plates.

ACCEPTANCE CRITERIA

i Test microorganisms must demonstrate a concentration range between $1.0x10^6$-1.0×10^7 cfu/mL (*S. aureus*) and greater than 1.0×10^5 conodia/mL (*T. interdigitale).*
ii Neutralizing subculture must provide growth when inoculated with <100 cfu/mL.
iii Sterility controls must produce no growth.
iv Viability controls must produce growth.
v Media sterility controls must produce no growth.
vi Test microorganism must demonstrate pure target organism.
vii Test substance must produce $\leq$ 3 positive carriers per 60 carriers tested (*S. aureus)* and 0 positive carriers per 10 carriers tested (*T. interdigitale)*.

Retest Guidance
i If inoculum concentration is $> 1.0 \times 10^7$ cfu/mL and all negative controls are acceptable, no retest is necessary.
ii If inoculum concentration is $<1.0 \times 10^5$ cfu/mL, retest is required.
iii If a test fails and inoculum concentration is $<1.0 \times 10^5$ cfu/mL, retest is not required.
iv If a test fails and inoculum is $>1.0 \times 10^7$ cfu/mL, retest may be conducted.
v If inoculum concentrations are within target range and all controls are acceptable, but test fails, retest may not be conducted.

1355 Pacific Place
Suite 101
Ferndale, WA 98248
phone: 360.733.1205
fax: 888-818-2978
email: lab@exactscientific.com

CALCULATIONS AND STATISTICAL ANALYSIS

The following calculations were used in this study to evaluate efficacy of testing procedures and product.

i cfu/mL of microbial counts: colony forming units * 10^{-x}, where x is the countable dilution used.
ii Log Calculation: Log_{10} (cfu/mL* 10^{-x})
iii Mean Log Density (LD): Average Log_{10} cfu/mL of sample set
iv Geometric Mean (GM): Antilog (Average Log_{10} cfu/mL) of sample set

STUDY RECORD AND TEST SUBSTANCE RETENTION

Study Record Retention
The original study report, protocol, and raw data will be maintained at Exact Scientific Services for a minimum of 5 years following the study completion date. After this retention time, records may be destroyed. Study sponsor may request copy of report at any time within the retention period.

All facility records pertaining to quality assurance and employee training will be maintained at Exact Scientific Services for a minimum of three years.

Test substance will be retained for a minimum of 30 days following the study completion date. Test substance may be returned to study sponsor upon request after this time. Arrangements may be made for extended storage period, if necessary, upon request.
s

1355 Pacific Place
Suite 101
Ferndale, WA 98248
phone: 360.733.1205
fax: 888-818-2978
email: lab@exactscientific.com

RESULTS

Neutralizer Subculture Study

Table 1: Neutralization Confirmation Study for *S. aureus*

Product Lot: 2020/12/07	Dilutions Tested			
Treatment	10^{-4}	10^{-5}	10^{-6}	10^{-7}
Product Test: Neutralizer Primary Subculture Treatment				
Primary Subculture Treatment	+	+	+	+
Confirmation				Confirmed
Product Test: Neutralizer Secondary Subculture Treatment				
Primary Subculture Treatment	+	+	+	+
Secondary Subculture Treatment	+	+	+	+
Confirmation				Confirmed
Inoculated Control: Neutralizer Primary Subculture Treatment				
Primary Subculture Treatment	+	+	+	+
Confirmation				Confirmed
Inoculated Control: Neutralizer Secondary Subculture Treatment				
Primary Subculture Treatment	+	+	+	+
Secondary Subculture Treatment	+	+	+	+
Confirmation				Confirmed
Negative Control: Neutralizer Primary Subculture Treatment				
Primary Subculture Treatment	-	-	-	-
Negative Control: Neutralizer Secondary Subculture Treatment				
Primary Subculture Treatment	-	-	-	-
Secondary Subculture Treatment	-	-	-	-
	Geometric Mean			
Inoculum Numbers Control	10^{-4}	10^{-5}	10^{-6}	10^{-7}
S. aureus	730	110	10	1
Confirmation			Confirmed	

1355 Pacific Place
Suite 101
Ferndale, WA 98248
phone: 360.733.1205
fax: 888-818-2978
email: lab@exactscientific.com

Table 2: Neutralization Confirmation Study for *T. interdigitale*

Product Lot: 2020/12/07	Dilutions Tested			
Treatment	10^{-4}	10^{-5}	10^{-6}	10^{-7}
Product Test: Neutralizer Primary Subculture Treatment				
Primary Subculture Treatment	+	+	+	+
Confirmation				Confirmed
Product Test: Neutralizer Secondary Subculture Treatment				
Primary Subculture Treatment	+	+	+	+
Secondary Subculture Treatment	+	+	+	+
Confirmation				Confirmed
Inoculated Control: Neutralizer Primary Subculture Treatment				
Primary Subculture Treatment	+	+	+	+
Confirmation				Confirmed
Inoculated Control: Neutralizer Secondary Subculture Treatment				
Primary Subculture Treatment	+	+	+	+
Secondary Subculture Treatment	+	+	+	+
Confirmation				Confirmed
Negative Control: Neutralizer Primary Subculture Treatment				
Primary Subculture Treatment	-	-	-	-
Negative Control: Neutralizer Secondary Subculture Treatment				
Primary Subculture Treatment	-	-	-	-
Secondary Subculture Treatment	-	-	-	-
	Geometric Mean			
Inoculum Numbers Control	10^{-4}	10^{-5}	10^{-6}	10^{-7}
T. interdigitale	70	30	10	2
Confirmation			Confirmed	

1355 Pacific Place
Suite 101
Ferndale, WA 98248
phone: 360.733.1205
fax: 888-818-2978
email: lab@exactscientific.com

Use Dilution Study

Table 3: Carrier Drying Conditions

Test Date	Test Organism	Test Substance Lot	Drying Time	Temperature
07/07/2021	*T. interdigitale*	2020/12/06	40 Minutes	24-26ºC
07/08/2021	*T. interdigitale*	2020/12/05		
07/21/2021	*S. aureus*	2020/12/05		35-37ºC
07/27/2021	*S. aureus*	2020/12/05		
07/28/2021	*S. aureus*	2020/12/06		
08/02/2021	*S. aureus*	2020/12/07		

Table 4: Inoculum Numbers Controls

Test Date	Test Organism	Test Substance Lot	Average Inoculum cfu/mL	Mean log_{10} Cell Density
07/07/2021	*T. interdigitale*	2020/12/06	$5.50x10^6$	6.74
07/08/2021	*T. interdigitale*	2020/12/05	$5.20x10^6$	6.72
07/21/2021	*S. aureus*	2020/12/05	$9.00 x10^5$	5.95
07/27/2021	*S. aureus*	2020/12/05	$1.03 x10^7$	7.01
07/28/2021	*S. aureus*	2020/12/06	$7.20 x10^6$	6.86
08/02/2021	*S. aureus*	2020/12/07	$9.00 x10^6$	6.95

Table 5: Use Dilution Method Results (Growth/No Growth)

Test Date	Test Organism	Test Substance Lot	Number Carriers Tested	Number Carriers with Growth	Number Carriers Confirmed
07/07/2021	*T. interdigitale*	2020/12/06	10	0	0
07/08/2021	*T. interdigitale*	2020/12/05	10	0	0
07/21/2021	*S. aureus*	2020/12/05	60	Retest due to unacceptable control results	
07/27/2021	*S. aureus*	2020/12/05	60	1	1
07/28/2021	*S. aureus*	2020/12/06	60	1	0
08/02/2021	*S. aureus*	2020/12/07	60	3	2

Table 6: Use Dilution Method Results (Reduction cfu/mL)

Test Date	Test Organism	Test Substance Lot	Number Carriers Tested	Average cfu/mL	Average Reduction cfu/mL
07/07/2021	*T. interdigitale*	2020/12/06	10	0.00	$5.50x10^6$
07/08/2021	*T. interdigitale*	2020/12/05	10	0.00	$5.20x10^6$
07/21/2021	*S. aureus*	2020/12/05	60	Retest due to unacceptable control results	
07/27/2021	*S. aureus*	2020/12/05	60	0.07	$1.03 x10^7$
07/28/2021	*S. aureus*	2020/12/06	60	0.00	$7.20 x10^6$
08/02/2021	*S. aureus*	2020/12/07	60	0.05	$9.00 x10^6$

1355 Pacific Place
Suite 101
Ferndale, WA 98248
phone: 360.733.1205
fax: 888-818-2978
email: lab@exactscientific.com

Table 7: Growth Confirmation

Test Date	Test Organism	Test Substance Lot	Colony Tested	Gram Stain	Selective Agar Morphology
07/07/2021	*T. interdigitale*	2020/12/06	Viability	Mold, filamentous	N/A
07/08/2021	*T. interdigitale*	2020/12/05	Viability	Mold, filamentous	N/A
07/27/2021	*S. aureus*	2020/12/05	Viability	Pos, cocci	Black with hemolytic zone
			Pos #1	Pos, cocci	Black with hemolytic zone
07/28/2021	*S. aureus*	2020/12/06	Viability	Pos, cocci	Black with hemolytic zone
			Pos #1	Mold	No Growth
08/02/2021	*S. aureus*	2020/12/07	Viability	Pos, cocci	Black with hemolytic zone
			Pos #1	Pos, cocci	Black with hemolytic zone
			Pos #2	Pos, cocci	Black with hemolytic zone
			Pos #3	Mold	No Growth

STUDY CONCLUSION

Test Substance Synergy Envirotab (lots: 2020/12/05, 2020/12/06, 2020/12/07) was tested against *Staphylococcus aureus* and *Trichophyton interdigitale*. A total of 60 inoculated carriers and 10 inoculated carriers for *S. aureus* and *T. interdigitale respectively* were exposed to each lot of the test substance with a contact time of 4 minutes. The carriers were then neutralized in letheen broth.

Following 4-minute contact time, Synergy Envirotab lot: 2020/12/05 disinfected 59 of 60 carriers; lot 2020/12/06 disinfected 60 of 60 carriers; and lot 2020/12/07 disinfected 58 of 60 carriers from *S. aureus*. Synergy Envirotab lot 2020/12/05 and lot 2020/12/06 disinfected 10 of 10 carriers from *T. interdigitale*.

Under the protocol conducted and the acceptance criteria provided, all lots tested meet requirements stated in the U.S. EPA Product Performance Test Guidelines OCSPP 810.2000: Disinfectants for Use on Environmental Surfaces Guidance for Efficacy Testing. The efficacy of Synergy Envirotab is not affected by the type of water used.

This study was conducted in compliance with the approved protocol, all experimental controls met the established acceptance criteria.

1355 Pacific Place
Suite 101
Ferndale, WA 98248
phone: 360.733.1205
fax: 888-818-2978
email: lab@exactscientific.com

REFERENCES

EPA MB-05-16 AOAC Use Dilution Method for Testing Disinfectants

EPA MB-22 Disinfectant Sample Preparation

EPA MB-03 Screening of Stainless Steel Penicylinders Used in Disinfectant Efficacy Testing

EPA MB-17 Neutralization Confirmation

U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines OCSPP 810.2000: Disinfectants for use on Environmental Surfaces Guidance for Efficacy Testing February 2018.

1355 Pacific Place
Suite 101
Ferndale, WA 98248
phone: 360.733.1205
fax: 888-818-2978
email: lab@exactscientific.com

APPENDIX 1

ESS 6.2.52 EPA Use Dilution Method Testing Standard Operating Procedures

Sections Included in this Document:

---

1. Purpose

1.1. The purpose of this test is to determine the efficacy of disinfectants against bacterial and/or fungal microorganisms.

2. Scope

2.1. This method applies to liquid-based disinfectants for use on hard surfaces.

3. Responsibilities

3.1. Analyst:

3.1.1. Follow this procedure

3.1.2. Enter results in Laboratory Information Management System (LIMS)

3.1.3. Submit a draft for review and approval

3.2. Director(s):

3.2.1. Review data

3.2.2. Approve data in LIMS

3.2.3. Submit results to client

4. Background

4.1. This method was developed by the United States Environmental Protection Agency (EPA) to determine efficacy of both dilutable and ready to use disinfectants for the purpose of certification. This method directly reflects EPA's method.

5. References

5.1. EPA MB-05-16 AOAC Use Dilution Method for Testing Disinfectants

5.2. EPA MB-22 Disinfectant Sample Preparation

5.3. EPA MB-03 Screening of Stainless Steel Penicylinders Used in Disinfectant Efficacy Testing

5.4. EPA MB-17 Neutralization Confirmation

6. Materials and Equipment

6.1. Instruments

6.1.1. Refrigerator (4 ± 2 °C)

6.1.2. Incubator (36 ± 1 °C)

6.1.3. Vortex

6.1.4. Sonicator

1355 Pacific Place
Suite 101
Ferndale, WA 98248
phone: 360.733.1205
fax: 888-818-2978
email: lab@exactscientific.com

6.1.5. Spectrophotometer

6.2. Equipment

6.2.1. Sterile glass tubes
6.2.2. Sterile pipettes (2 mL, 10 mL, 25 mL)
6.2.3. Sterile petri dishes
6.2.4. Sterile #2 filter paper
6.2.5. Lighter and metal forceps
6.2.6. Digital timer
6.2.7. Thermometers
6.2.8. Pipette bulb or equivalent
6.2.9. Glass beaker (1 L, 500 mL)
6.2.10. Sterile stainless steel penicylinders (80 minimum)

6.2.10.1. Carriers, polished stainless steel penicylinders for the AOAC Use-dilution Method. Polished stainless-steel cylinders, 8 ± 1 mm outer diameter, 6 ± 1 mm inner diameter, 10±1 mm length; type 304 stainless steel, SS 18-8 (S & L Aerospace Metals, or Fisher Scientific Cat. No. 07-907-5Q).

6.3. **Reagents**

6.3.1. Phosphate Buffered Water (PBW)
6.3.2. Letheen Broth with Tween 80 (LB)
6.3.3. Tryptic Soy Broth (TSB)
6.3.4. Sabbourd Dextrose Agar (SabDex)
6.3.5. Blood Agar with Esculin (BEA)
6.3.6. 70% EtOH
6.3.7. DI Water, Sterile and Non-Sterile
6.3.8. Disinfectant

6.3.8.1. BTC 835:50 % n-alkyl (50 % C14, 40 % C12, 10 % C16) Dimethyl Benzyl Ammonium Chloride

6.3.9. Sodium Hydroxide (NaOH) 1N
6.3.10. Phenolphthalein 1 % (w/v) solution in alcohol
6.3.11. Test Product- prepared according to manufacturer's instructions

7. Procedure

7.1. **Carrier Screening**

This procedure describes the preparation of stainless steel penicylinder carriers for the AOAC Use-Dilution Method. This procedure is applicable to stainless steel penicylinder carriers only. For any alternative carrier, see EPA SOP MB-03-07.

7.1.1. Physical Screening

7.1.1.1. Visually screen polished stainless-steel carriers.
7.1.1.2. Discard carriers that fail physical screening due to visible damage (dull, chipped, dented, or gouged).
7.1.1.3. Place carriers that pass physical screening in a container and label with date and "Physically Screened"

7.1.2. Cleaning

7.1.2.1. Soak the physically screened carriers overnight (approx. 12 hr) in 1N NaOH.
7.1.2.2. Rinse several (3-4) times with tap water.
7.1.2.3. Collect a portion of the last rinse water and add 2-3 drops of 1% phenolphthalein.
7.1.2.4. If any NaOH remains, the phenolphthalein turns pink, indicating the need for additional rinsing. Continue to rinse the carriers until the addition of phenolphthalein to the collected portion of the rinse water does not produce a color change (to pink).

1355 Pacific Place
Suite 101
Ferndale, WA 98248
phone: 360.733.1205
fax: 888-818-2978
email: lab@exactscientific.com

7.1.2.5. Rinse twice more with DI water. Allow carriers to air dry and store in a closed container marked with date and "Cleaned Carriers/Not Biologically Screened."

7.1.3. Biological Screening

7.1.3.1. Place the cleaned carriers into 25×150 mm test tubes, 20 per tube.

7.1.3.2. Cover the carriers with DI water and cap.

7.1.3.3. Autoclave at 121 ºC for 20 min; cool and store at room temperature.

7.1.3.4. Perform Use-Dilution testing (see section 7.5) on each carrier using the following parameters:

7.1.3.5. *Staphylococcus aureus* and/or *Trycophyton interdigitale*, 500 ppm solution of BTC 835 prepared using sterile deionized water, 20±1 ºC, no organic soil, ten-minute exposure period, and letheen broth as the neutralizer. Use primary subculture tubes only.

7.1.3.6. Select Control Carriers

a. Select one (1) carrier from each petri dish for controls.
b. Three (3) pre-carrier controls- used to enumerate initial carrier bacterial concentrations.
c. Three (3) post-carrier controls- used to enumerate carrier bacterial concentrations upon method completion.
d. One (1) viability control carrier- untreated, inoculated carrier.

7.1.3.7. Pre-Carriers: Aseptically transfer three carriers to sterile tubes with 10mL of letheen broth.

a. Sonicate for one minute according to diagram 1 in appendix. Make sure water and letheen broth levels are even.
b. After sonication, mix and make four ten-fold serial dilutions into 9 mL PBS tubes.
c. Plate dilutions 10-1, -3, -5 in duplicate.
d. Incubate plates at 36±1ºC for 48±2 hours.

7.1.3.8. Post-Carriers: Aseptically transfer three carriers to sterile, empty tubes for later use.

a. Upon study completion, repeat steps for pre-carriers above.

7.1.3.9. Viability Control: Aseptically transfer one inoculated carrier into tube containing 10mL letheen broth.

a. Keep control at 2-5 °C until study is complete. Incubate tube with all other tubes.

7.1.3.10. Autoclave and repeat procedures in section 7.1 for all failed carriers.

7.1.3.11. Autoclave all passing carriers separately.

7.1.3.12. Re-wash according to procedure 7.12.

7.1.3.13. These carriers are to be collected.

a. After drying, store in sterile, sealed container marked as "Sterile Pooled Carriers".

7.1.4. Preparing for UDM Testing

7.1.4.1. Remove required number of cleaned carriers from the "Sterile Pooled Carriers".

7.1.4.2. Place carriers into 25x150mm test tube (20 per tube).

7.1.4.3. Cover carriers in tubes with DI water.

7.1.4.4. Autoclave at 121 °C for 20 minutes.

7.1.4.5. Cool and store at room temperature.

7.1.4.6. After UDM testing, all negative carriers and those used as controls, clean according to section7.1.2 and return to master pool.

1355 Pacific Place
Suite 101
Ferndale, WA 98248
phone: 360.733.1205
fax: 888-818-2978
email: lab@exactscientific.com

a. Any carriers giving positive result in UDM, must be re-screened according to section 7.1.

7.2. **Inoculum Preparation**

This procedure describes how to prepare the inoculum to be used in any of the methods in this procedure.

7.2.1. ATCC Culture Maintenance

7.2.1.1. ATCC microorganism strains are to be obtained in either Kwik Stik dehydrated pellets or freeze-dried dehydrated pellet vials.

7.2.1.2. Organisms are to be logged into the Microorganism Logbook to record received date, organism, ATCC number, lot number, expiration date, and hydration date.

7.2.1.3. Once re-hydrated per manufacturer instructions, all bacterial microorganisms are to be plated onto BEA to ensure purity and incubated in TSB at 35 °C (or appropriate temperature) for 24 hours.

7.2.1.4. Bacterial Cultures may be stored in TSB and/or isolated onto BEA and wrapped in parafilm for one month in refrigerated conditions (4 ± 2 °C).

7.2.1.5. Fungal cultures are to be plated onto SabDex after re-hydration per manufacturer instructions and incubate at ambient temperatures (25 ± 2 °C) for 5-10 days as appropriate per organism.

7.2.1.6. Fungal cultures may be stored at room temperature wrapped in parafilm up to two weeks.

7.2.2. *Staphylococcus aureus* ATCC 6538

7.2.2.1. From culture plate, use a sterile swab to harvest colonies and disperse into 9 mL PBW.

7.2.2.2. Using a spectrophotometer, set to read percent transmittance at 530 nm wavelength.

7.2.2.3. Dilute or concentrate bacterial suspension as appropriate to obtain 78-80 % transmittance

a. This is an estimated cell concentration of 1.0×10^9 cfu/mL.

7.2.2.4. Use serial dilutions into 9 mL PBW to obtain target concentration as described in the method.

7.2.3. *Trichophyton interdigitale* ATCC 9533

7.2.3.1. From culture plate, use a sterile swab to harvest colonies and disperse into 9 mL PBW.

7.2.3.2. Using a spectrophotometer, set to read percent transmittance at 530 nm wavelength.

7.2.3.3. Dilute or concentrate bacterial suspension as appropriate to obtain 23-25 % transmittance

a. This is an estimated cell concentration of 1.0×10^9 cfu/mL.

7.2.3.4. Use serial dilutions into 9 mL PBW to obtain target concentration as described in the method.

7.3. **Product Preparation**

This procedure describes the preparation of the product to be used in the Use Dilution Method. This can be prepared according to the directions on the client supplied product. Sterile water or AOAC Synthetic Hard Water (see 7.3.3) may be used depending on the product. AOAC Synthetic Hard Water Solution 1 and 2 (see 7.3.1 and 7.3.2) must be prepared to make the AOAC Synthetic Hard Water.

7.3.1. AOAC Synthetic Hard Water Solution 1

7.3.1.1. Dissolve 7.94 g $MgCl_2$ (anhydrous) and 18.50 g CaCl2 in boiled deionized water and bring to a volume of 250 mL volumetrically.

1355 Pacific Place
Suite 101
Ferndale, WA 98248
phone: 360.733.1205
fax: 888-818-2978
email: lab@exactscientific.com

7.3.1.2. Sterilize by membrane filtration. Used for the preparation of hard water at various concentrations.

7.3.2. AOAC Synthetic Hard Water Solution 2

7.3.2.1. Dissolve 14.01 g $NaHCO_3$ in boiled deionized water and bring to a volume of 250 mL volumetrically.

7.3.2.2. Sterilize by membrane filtration. Used for the preparation of hard water at various concentrations.

7.3.3. Preparation of 400 ppm AOAC Synthetic Hard Water

7.3.3.1. Per 1 L: Add 4 mL of AOAC Synthetic Hard Water Solution 1 (1 mL for each 100-ppm hardness desired) and 4 mL of AOAC Synthetic Hard Water Solution 2 to a 1L volumetric flask and bring to volume with sterile deionized water.

a. If preparing concentration other than 400 ppm, per 1 L preparation, use 1mL of AOAC Synthetic Hard Water Solution 1 (per 100 ppm hardness desired) and 4 mL AOAC Synthetic Hard Water solution 2.
b. Bring to volume using sterile deionized water in a 1 L volumetric flask and continue with steps 7.3.3.2 and 7.3.3.3 below.

7.3.3.2. Measure the pH of the hard water sample. The pH should be between 7.6 and 8.0 at room temperature. If necessary, adjust the pH using 1 N NaOH or 1N HCl.

7.3.3.3. Filter sterilize the hard water using a 0.2 µm filter unit.

7.4. **Neutralizing Confirmation**

This procedure describes the evaluation of the effectiveness of neutralizers specified for disinfectant UDM testing. This is a quantitative approach to determine the effectiveness of the neutralizer itself to neutralize the effects of the disinfectant product being tested, using the same conditions specified for product testing (water hardness, pH, neutralizer, contact time, temperature). It is imperative that this method be performed prior to UDM testing as the product tested must be completely neutralized post exposure time.

For this method, use pre-screened polycylinders from the sterile master pool. You will need 8 test carriers, and a negative carrier control per test organism.

7.4.1. Product Preparation

7.4.1.1. Prepare product according to section 7.3.

7.4.1.2. Use for testing within three hours of preparation.

7.4.2. Inoculum Preparation

7.4.2.1. Prepare inoculum according to section 7.2.2 (*S. aureus)* or 7.2.3 (*T. interdigitale*) to obtain a culture suspension of approximately 1.0 x 10^9 cfu/mL.

7.4.2.2. Prepare ten-fold serial dilutions by inoculating 1 mL of test culture into 9 mL of PBW, 7 times. Use the 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} dilutions for this method.

7.4.2.3. Obtain numbers control by plating 0.1 mL of each of the four dilutions in duplicate on TSA. Incubate plates at 36 ± 1 °C for 48 ± 2 hours.

7.4.2.4. Use within 30 minutes or refrigerate at 4 ± 1 °C for up to two hours.

7.4.3. Neutralizing Subculture Procedures

7.4.3.1. Start with a total of 38 tubes containing 10mL each of neutralizing broth:

a. 4 – Primary Subculture Treatment
b. 8 – Secondary Subculture Treatment
c. 4 – Positive Primary Subculture Control
d. 8 – Positive Secondary Subculture Control
e. 4 – Negative Primary Subculture Control
f. 8 – Negative Secondary Subculture Control

1355 Pacific Place
Suite 101
Ferndale, WA 98248
phone: 360.733.1205
fax: 888-818-2978
email: lab@exactscientific.com

7.4.3.2. Apply product to 8 test carriers according to the specific instructions provided by the manufacturer (e.g. Use dilution, application, contact time, etc.).
   a. Use 4 test carriers per product, per one test organism for each of the primary and secondary subculture treatment.
   b. Be sure to start contact time at 30s- or 60s-time intervals to allow enough time for transfer.

7.4.3.3. After specified contact time, aseptically remove carrier from product and allow to drain, in order so that each carrier has been exposed to product for the exact specified time.

7.4.3.4. Place carrier in a timed fashion into neutralizing broth.

7.4.3.5. Incubate at room temperature for 30-45 minutes.

7.4.3.6. Put aside the 4 primary test carriers.

7.4.3.7. In order, Transfer each of the secondary subculture carriers to the second tube containing 10 mL neutralizing broth. This step is not timed.

7.4.4. Controls

7.4.4.1. Uninoculated Controls
   a. Neutralizer-primary and secondary subculture negative controls: a minimum of 1 tube each of uninoculated neutralizer and secondary subculture media will be included in the test and incubated with the other tubes.
   b. Negative Carrier: uninoculated carrier in 10 – 20 mL of TSB or Letheen broth incubated at 36 ± 1 °C for 3 - 10 days.

7.4.4.2. Inoculated Controls
   a. Neutralizer-primary positive control: four tubes of fresh neutralizer, unexposed to disinfectant, one tube for each inoculum dilution.
   b. Neutralizer-secondary positive control: four tubes of secondary subculture media, unexposed to disinfectant, one tube for each inoculum dilution.

7.4.5. Treatment Inoculation

7.4.5.1. After step 7.4.3.7, inoculate all tubes concurrently using Table 1 below.

Table 1

	Dilutions Added			
Treatment	**10^{-4}**	**10^{-5}**	**10^{-6}**	**10^{-7}**
Product Test: Neutralizer Primary Subculture Treatment				
Primary Subculture Treatment	0.1mL	0.1mL	0.1mL	0.1mL
Product Test: Neutralizer Secondary Subculture Treatment				
Primary Subculture Treatment	0.1mL	0.1mL	0.1mL	0.1mL
Secondary Subculture Treatment	0.1mL	0.1mL	0.1mL	0.1mL
Inoculated Control: Neutralizer Primary Subculture Treatment				
Primary Subculture Treatment	0.1mL	0.1mL	0.1mL	0.1mL
Inoculated Control: Neutralizer Secondary Subculture Treatment				
Primary Subculture Treatment	0.1mL	0.1mL	0.1mL	0.1mL
Secondary Subculture Treatment	0.1mL	0.1mL	0.1mL	0.1mL

1355 Pacific Place
Suite 101
Ferndale, WA 98248
phone: 360.733.1205
fax: 888-818-2978
email: lab@exactscientific.com

Negative Control: Neutralizer Primary Subculture Treatment				
Primary Subculture Treatment	N/A	N/A	N/A	N/A
Negative Control: Neutralizer Secondary Subculture Treatment				
Primary Subculture Treatment	N/A	N/A	N/A	N/A
Secondary Subculture Treatment	N/A	N/A	N/A	N/A

7.4.5.2. Shake tubes thoroughly.
7.4.5.3. Incubate all tubes at 36 ± 1 °C for 48 ± 2 hours.
7.4.6. Results and Confirmation
7.4.6.1. Record results as "+" for turbidity and "-" for no growth.
7.4.6.2. For each treatment and control group, confirm a minimum of one positive tube per treatment as stated below. Select the tube with the highest dilution showing growth to confirm.
a. *S. aureus*: gram stain and culture on Baird Parker Agar. Should be gram positive cocci under microscope. Selective agar should show black colonies with hemolytic zones.
b. *T. interdigitale*: filamentous mold under microscope.
7.4.7. Interpretation
7.4.7.1. Numbers Control
a. One of the four dilutions should provide counts within 50-100 cfu/mL range.
7.4.7.2. Controls
a. Negative controls should show no growth. Growth in this case indicates sterility issues and the test will need to be repeated. Positive controls should show consistent growth among primary and secondary neutralizing broths. If growth rates differ greatly, and/or if no growth is observed in tubes with high level of inoculum, this indicates poor media performance and alternative media may be necessary.
7.4.7.3. Treatments
a. If growth in primary treatment tubes, only one neutralization step is necessary. If no growth is observed in primary treatment, but growth is observed in secondary treatment, primary neutralization is not sufficient and secondary transfer is necessary for UDM. If no growth is observed in either primary or secondary tubes, an alternative neutralizing broth is necessary and test should be repeated.

7.5. **Use Dilution Method**

This procedure describes the efficacy determination of liquid-based disinfectants against *S. aureus* and *T. interdigtale* on hard surfaces. Stainless steel polycylinders are inoculated and exposed to test product.
Prior to conducting UDM, perform all procedures above in section 7 for each product. All tests must provide acceptable results before continuing with UDM procedures.
For *S. aureus* a minimum of 3 different product lots are to be evaluated using 60 test carriers on 3 different days. For *T. interdigitale*, a minimum of 2 different product lots are to be evaluated using 10 test carriers on 2 different days.

7.5.1. Inoculum Preparation

1355 Pacific Place
Suite 101
Ferndale, WA 98248
phone: 360.733.1205
fax: 888-818-2978
email: lab@exactscientific.com

7.5.1.1. Prepare inoculum according to section 7.2.2 (*S. aureus*) or 7.2.3 (*T. interdigitale*) to obtain a culture suspension of approximately 1.0 x 10^9 cfu/mL.

7.5.1.2. Target Dilutions: *S. aureus*: Final mean log density 6.0-7.0 (geometric mean density of $1.0x10^6$ - $1.0x10^7$)

a. Transfer 2 mL of initial $1.0x10^9$ suspension into 198 mL PBS for final $1.0x10^7$ cfu/mL inoculum.
b. Plate numbers control from 200 mL inoculum (concentration $1.0x10^7$) using 10^{-1}, $^{-3}$, $^{-5}$ in duplicate.

7.5.1.3. Target Dilutions: *T. interdigitale*: > $5.0x10^6$ conidia/mL

a. Transfer 1 mL of initial $1.0x10^9$ suspension into 9 mL PBS for $1.0x10^8$ conidia/mL suspension.
b. Transfer 1 mL of suspension into 9 mL PBS for final $1.0x10^7$ conidida/mL suspension
c. Plate numbers control from 200 mL inoculum (concentration $1.0x10^7$) using 10^{-1}, $^{-3}$, $^{-5}$ in duplicate.

7.5.1.4. Use inoculum within 30 minutes or refrigerate at 4 ± 1 °C for up to two hours.

7.5.2. Inoculating Carriers

7.5.2.1. Before inoculating carriers, remove negative control carriers.

a. One (1) Negative Carrier- untreated, uninoculated. Aseptically transfer to tube with 10 mL letheen broth.
   i. Keep refrigerated at 2 – 5 °C until study is completed. Incubate with all other letheen broth tubes.
b. One (1) Negative Process Control- treated, uninoculated. Aseptically transfer to sterile, empty tube until ready to use.

7.5.2.2. Pipette 20 mL of final inoculum suspension from section 7.5.1 into sterile 25x150 mm test tubes containing 20 sterile, dry carriers each.

a. Inoculate 80 total carriers for *S. aureus*.
b. Inoculate 40 total carriers for *T. interdigitale*.

7.5.2.3. Incubate at room temperature for 15 ± 2 minutes. Aseptically transfer carriers from inoculum to sterile petri dishes lined with two layers of sterile #2 filter paper. Place lid on dish.

7.5.2.4. Place no more than 12 carriers per petri dish.

a. Carriers must be standing upright and should not fall over or touch the side of the petri dish.
b. Dry carriers at 36 ± 1 °C for 40 ± 2 minutes.
   i. All carriers must be exposed to disinfectant within 2 hours of drying.

7.5.3. Selecting Control Carriers

7.5.3.1. Select 1 carrier from each petri dish for controls.

a. Three (3) pre-carrier controls- used to enumerate initial carrier bacterial concentrations.
b. Three (3) post-carrier controls- used to enumerate carrier bacterial concentrations upon method completion.
c. One (1) viability control carrier- untreated, inoculated carrier.

7.5.3.2. Pre-Carriers: Aseptically transfer three carriers to sterile tubes with 10 mL of letheen broth.

a. Sonicate for 1 minute according to diagram 1 appendix. Make sure water and letheen broth levels are even.
b. After sonication, mix and make 4 ten-fold serial dilutions into 9 mL PBS tubes.

1355 Pacific Place
Suite 101
Ferndale, WA 98248
phone: 360.733.1205
fax: 888-818-2978
email: lab@exactscientific.com

c. Plate dilutions 10^{-1}, $^{-3}$, $^{-5}$ in duplicate.
d. Incubate plates at 36 ± 1 °C for 48 ± 2 hours.

7.5.3.3. Post-Carriers: Aseptically transfer three carriers to sterile, empty tubes for later use.
a. Upon study completion, repeat steps 7.5.3.2 for pre-carriers.

7.5.3.4. Viability Control: Aseptically transfer one inoculated carrier into tube containing 10mL letheen broth.
a. Keep control at 2 – 5 °C until study is complete. Incubate tube with all other tubes.

7.5.4. Sample Test Procedure

7.5.4.1. Prepare sample according to manufacturer instructions and section 7.3.
a. Dispense 10 mL product into sterile tubes, 1 tube per carrier.
b. Allow tubes to equilibrate at least 10 minutes to 20 ± 1 °C.

7.5.4.2. Aseptically and sequentially transfer carriers from petri dish to tubes containing 10mL disinfectant product at 30 second or 60 second intervals.
a. Add one carrier to a product tube and swirl 2-3 times before placing back into water bath.
b. Add carrier within ±5 seconds of specified time.
c. Use alternating sterile hook or equivalent to transfer carriers. Flame sterilize the hook between carriers.
d. Be sure to include negative process control in this step. Treat this carrier as all other carriers from this point on.

7.5.4.3. Following exposure time, sequentially transfer carriers into neutralizer media (letheen broth or equivalent).
a. Remove carrier and tap along interior of tube to remove excess disinfectant.
b. Transfer with ±5 seconds of specified time.
c. If secondary neutralizing step is necessary, incubate at room temperature for 30-45 minutes and transfer to secondary neutralizing broth as performed in section 7.4.

7.5.4.4. Once all carriers have been transferred, sonicate letheen broth according to pre/post carrier instructions in section 7.5.3.
a. Plate all carrier tubes using 1 mL in duplicate.

7.5.4.5. Incubate letheen broth tubes at 36 ± 1 °C for 48 ± 2 hours.

7.5.5. Reusing Carriers

7.5.5.1. All carriers that were negative should be autoclaved and re-sterilized to be added to the sterile master pool.

7.5.5.2. All positive carriers should be autoclaved and perform biological screening in section 7.1.

8. Records and Results

8.1. Results and Interpretation

8.1.1. Record all letheen broth tubes as "+" with turbidity or "-" with no growth.

8.1.2. Record all colony counts in cfu/mL or conidia/mL as appropriate.

8.1.3. Viability Controls: Growth should be observed. If no growth is observed, repeat the test.

8.1.4. Sterility Controls: If negative carrier or negative process carrier show growth, issues in sterility is indicated. The test must be repeated.

8.1.5. Test Carriers: Record results for test carriers. Acceptance criteria is as follows:

8.1.5.1. *S. aureus*: 0-3 positive carriers per 60 test carriers.
a. If positive carriers are observed, confirm a minimum of 4 positive carriers per test using gram stain and selective agar (per section 7.4.6.2.a).

1355 Pacific Place
Suite 101
Ferndale, WA 98248
phone: 360.733.1205
fax: 888-818-2978
email: lab@exactscientific.com

8.1.5.2. *T. interdigitale*: 0 positive carriers per 10 test carriers (in 10 minutes exposure time).
a. If positive carriers are observed, confirm using microscopic examination (per section 7.4.6.2.b).

8.2. Calculations
8.2.1. All data should be calculated in geometric mean cfu/mL or conidia/mL as appropriate, as well as average $\log_{10}$ cfu/mL.
8.2.2. Reduction may be calculated based on initial inoculum concentration determined by the numbers control and the final colony counts for each tube after sonication.

8.3. A final, signed report will be issued to the client. A copy of all record sheets and final report will be retained in accordance to ESS policy 1.6.8 Records and Data Management. All reagents and medias shall be traceable. All timing, temperatures, and procedures shall be documented. All quality control measures shall be documented.

9. Quality Control

Studies must include the following controls (as specified in each section above).

9.1. Carrier Sterility Control
9.1.1. For each test lot, an uninoculated carrier was collected and incubated in 10 mL of letheen broth alongside all test carriers.
9.1.2. Viability Control
9.1.2.1. For each test lot, an inoculated, untreated carrier was collected and incubated in 10 mL of letheen broth alongside all test carriers.
9.1.3. Negative Process Control
9.1.3.1. For each test lot, an uninoculated carrier was treated alongside test carriers to ensure no contamination occurred during any test procedures.
9.1.4. Neutralization Subculture Sterility Controls
9.1.4.1. Four primary neutralizing subculture media tubes and four secondary neutralizing subculture media tubes were incubated alongside test carriers.
9.1.5. Media Sterility Controls
9.1.5.1. All media lots went through quality control testing including inoculation with test organisms to ensure growth promotion, sterility evaluation, and pH monitoring.
9.1.6. Test Microorganism Purity Control
9.1.6.1. Test cultures used in this study were subcultured onto blood agar *(S. aureus*) or SabDex agar (*T. interdigitale*) and visually examined for purity prior to evaluation.
9.1.7. Confirmation of Positive
9.1.7.1. Positive tubes with turbidity/growth were confirmed via microscopic exam and/or colony morphology on selective agar plates.

10. Definitions

10.1. Carrier- A surrogate surface or matrix that facilitates the interaction of test microorganisms and treatments
10.2. CFU- Colony forming unit.
10.3. Efficacy- the proven performance of a product established under defined conditions of testing.
10.4. Geometric mean (GM)- the average microorganism count (cfu/mL) of replicates tested, calculated from logarithmic values: Antilog (Average Log_{10}(cfu/mL)).
10.5. Hard water- water which contains a standardized concentration of calcium and magnesium ions.
10.6. Inoculum- the viable microorganisms used to contaminate a sample, device, or surface, often expressed as to number and type.
10.7. Lower Control Limit (LCL)- lowest concentration of product considered out of statistical control due to special cause variation.

1355 Pacific Place
Suite 101
Ferndale, WA 98248
phone: 360.733.1205
fax: 888-818-2978
email: lab@exactscientific.com

10.8. Mean Log Density (LD)- average $\log_{10}$ converted carrier count cfu/mL of data per test day.
10.9. Neutralization- the process for inactivating or quenching the activity of a microbiocide, often achieved through physical or chemical means.
10.10. Primary Subculture- part of the neutralizing confirmation procedures, the initial exposure to the intended neutralizing liquid to be verified.
10.11. Primary Neutralizer- the neutralizing liquid carriers are initially exposed to, after being treated with disinfectant/sanitizer.
10.12. Sanitizer- chemical or physical agent(s) used to reduce the number of microorganisms to a level judged to be appropriate for a defined purpose and/or claim.
10.13. Secondary Subculture- part of the neutralizing confirmation procedures, after treated carrier is exposed to primary neutralizer, this is the subsequent exposure to intended neutralizing liquid to be verified.
10.14. Secondary Neutralizer- the neutralizing liquid carriers are exposed to after the primary neutralizing liquid. This can be the same neutralizing liquid used in the primary subculture or an alternative neutralizing liquid.
10.15. Test substance- an antimicrobial formulation used in testing.

11. Safety

11.1. Use appropriate PPE for all testing. Lab coat, safety goggles, and gloves are required for all methods listed above due to the unknown formulation and safety hazards of test substances.
11.2. Perform test product preparation (section 7.3) in a hood due to product off gassing when hydrated.

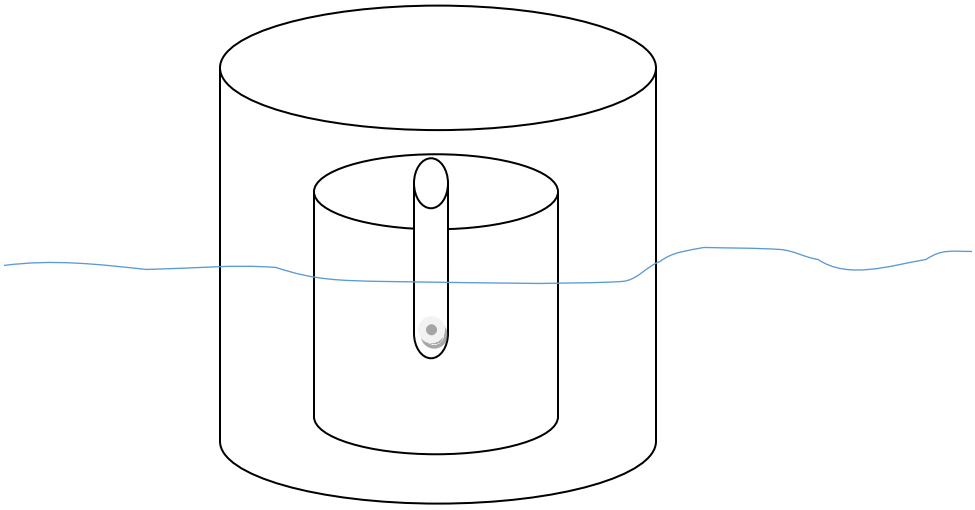
12. Attachments

12.1. None

13. Appendices

Diagram 1: Sonication Process

Ensure water/media levels are even and that both test tube and inner beaker do not touch the bottom of the sonicator nor the sides of outer containers.



Exact Scientific Services, Inc.

1355 Pacific Place
Suite 101
Ferndale, WA 98248

Phone: (360) 733-1205
Fax: (888) 818-2978
Email: lab@exactscientific.com

Certificate of Analysis

Client: Synergy Americas, Inc
127 Stream Road
Winterport, ME 04496

Phone: 207.944.3495

Email: michael@synergy-americas.com

Invoice Number: 21-13177
PO Number: EPA-GLP3
Received Date: 8/9/2021
Approved By:
Approved Date: 11/1/2021
Project Name: Use Dilution Test

Lab #: 53261 **Sample: Synergy Envirotab 4g Lots: 2020/12-05, 2020/12-06, 2020/12-07**

Analyte	Results	Units	Detection Limit	Method	Analyst	Date Analyzed
Administration						
Katherine	See Word Doc	PSU		ESS_1.1.27	KS	10/22/2021

ISO 17025 ANAB Accredited Laboratory Cert. #: AT-1754
I = ISO 17025 accredited method
ND = Not detected above the listed detection limit
MM = Modified Method
RR = Revised Result
SS = Analysis was run on a separate submission

This report and its results only relate to the specific sample(s) tested as received, and is for exclusive use of the customer named above and shall not be reproduced except in full.